REMARKS

Claims 1-19 are currently pending in the application. Claims 11 and 17 are amended. The amendments find support in the specification and are discussed in the relevant sections below. No new matter is added.

Claim Rejections 112

Claims 1-3 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant recognizes as the invention.

The office action states that the amendment to claim 1 establishes that Rep protein can initiate rolling circle replication. Applicant agrees with the following statement in the Office action: "that as claim 1 is written, the method comprises introducing a first and second vector into a prokaryotic host which expresses a gene encoding a site specific recombinase and a gene encoding a re protein to initiate rolling circle replication".

However the office action asserts that as "claim 1 is written, it is unclear how Rep-initiated rolling circle replication on two separate vectors (i.e. the first and second vector) would produce the product vector as claimed", and also states that the "further limitation of rolling circle replication by the rep protein still does not provide a functional link between the Rep protein and formation of a product vector from the first and second vector".

Before addressing the mechanism of formation of the vector product recited in claim 1, Applicant notes that in this method claim, the step of introducing the first and second vectors recited in claim 1 into a prokaryotic host cell which expresses site specific recombinase and Rep protein as required by claim 1, will result in the formation of a product vector which contains a gene of interest, the gene of interest having been transferred from the recited first vector, the gene of interest being interposed between the double-stranded origin of replication of the second vector and the site specific recombination site, as well as the other recited characteristics required of the product vector by claim 1.

The mechanism involved in the *in vivo* formation in a prokaryotic host of the product vector recited in claim 1 involves several steps as described in detail in the specification. First, the site specific, recombinase mediated, recombination of vector 1 and vector 2 at their respective site-specific recombination sites *in vivo* results in the formation of a cointegrate

plasmid as described on page 26, third paragraph, as diagrammed in Figure 1, and as described on page 20 of the instant specification:

> "The plasmids of the present invention comprise either a gene of interest or a negative selectable marker interposed between a double-stranded origin of replication and a site-specific recombination recognition site. The precise fusion between the first and second vector is catalyzed by a site specific recombinase. Site-specific recombinases are enzymes that recognize a specific DNA site or sequence termed a site-specific recombination recognition site, and catalyzes the recombination of DNA in relation to these sites."

After the formation of the cointegrate plasmid through site specific, recombinase mediated recombination of vector 1 and vector 2, the cointegrate plasmid then undergoes rolling circle replication initiated by the binding of the Rep protein to a double stranded origin of replication. As described in the final paragraph on page 9 of the specification, rolling circle replication in a typical vector with one double strand origin of replication as follows:

> "**DNA synthesis initiates at a double stranded origin of replication** from which a sole replication fork **proceeds around the template nucleic acid.** As the fork revolves, the newly synthesized strand displaces the previously synthesized strand from the template, producing a characteristic tail comprised of single stranded DNA. **The displaced strand is released from the plasmid once the replication fork encounters the double stranded origin of replication,** recircularized and may then be made double stranded via DNA synthesis which initiates from the single stranded origin of replication and processed into singular or multimeric copies of the original DNA.". (emphasis added)

However, the cointegrate plasmid has two double stranded origins of replication-one from vector 1 and one from vector 2. As stated on page 15, last sentence through first two lines of page 16 of the instant specification, a **key feature of the present invention**

> ".is that the *in vitro* and *in vivo* replication of a plasmid containing two double stranded origins of replication on the same strand lead to the formation of two smaller plasmids corresponding to the sequences located between the two double stranded origins of replication".

In the above passage from the specification, "a plasmid containing two double stranded origins of replication on the same strand" is referring to the cointegrate plasmid. And the product vector recited in claim 1 is one "of two smaller plasmids corresponding to the sequences located between the two double stranded origins of replication" of the cointegrate vector.

When rolling circle replication of the cointegrate initiates from the double stranded origin of replication adjacent to the single-stranded origin of replication as illustrated in Figure 1, the replication fork proceeds along the template comprising the single-stranded origin of replication, the selectable marker of the second vector (i.e. marker B in Figure 1), and the gene

of interest, until it arrives at the next double stranded origin of replication, where an incision is made resulting in the formation of a single stranded circular molecule consisting of the displaced strand, as described on page 28 of the specification. The displaced strand of this single stranded molecule (product vector of claim 1) comprises the single stranded origin of replication of the second vector, the marker of the second vector, the gene of interest and the double stranded origin of replication of the second vector, and the site specific recombination site, but does not include the selectable marker of the first vector, nor the negative selectable marker of the second vector.

Though not a requirement of the claim, the newly formed strand is made double stranded using its single strand origin of replication as described on page 16, last paragraph:

> "Replication of the single stranded DNA released upon completion of leading strand synthesis initiates from the plasmid single stranded origin of replication and is carried out solely by the proteins in the host cell".

In view of the comments above, Applicant submits that the method of claim 1 of introducing the first and second vectors containing the limitations recited in claim 1 into a prokaryotic host cell which expresses site specific recombinase and Rep protein as required by claim 1, will result in the formation of a product vector which contains a gene of interest, as well as the other recited characteristics required of the product vector by claim 1, rendering the claimed subject matter particularly pointed out and distinctly claimed. In view of these comments, Applicant respectfully requests reconsideration and withdrawal of this rejection of claim 1 and its dependent claims 2 and 3.

New Claim Rejections

Claim 11 is newly rejected as being indefinite because it recites the phrase "a second selectable marker" when there is no other selectable marker recited in this independent claim.

Accordingly Applicant has amended claim 11 by deleting the word "second", thus removing the claim's indefiniteness. .

Claim 17 is newly rejected as being indefinite in its recitation of the phrase "comprising a secondary host cell" in view of the fact that claim 17 is dependent on claim 12 or 13, neither of which recite a host cell. The Office action notes that claim 14, which

is dependent on claims 12 or 13, recites the limitation of a kit comprising a primary host cell.

Accordingly, Applicant has amended Claim 17 to depend from claim 14, thus removing the indefiniteness of claim 17.

In view of the amendments to claims 11 and 17, reconsideration and withdrawal of their rejection is respectfully requested.

Allowable Claims

Applicant acknowledges with appreciation the statement in the Office action that claims 4-10, 12-16 and 18-19 are allowed.

Conclusion

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

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